A Research Proposal

on

# Pharmaceutical and Preclinical Evaluation of *Reetha Bhasma* in the Management of Iron Deficiency Anemia

Submitted to

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# INTRODUCTION

## 1.1. Iron deficiency anemia (IDA)

Iron deficiency anemia (IDA) is a common type of anemia that occurs when iron loss (often from intestinal bleeding or menses) occurs, and/or the dietary intake or absorption of iron is insufficient. In such a state, hemoglobin, which contains iron, cannot be formed. IDA is one of the most common nutritional disorders world-wide, especially in India and other developing countries. Young children and women in the reproductive age group are the most vulnerable to iron deficiency anemia[1]. Surveys in different parts of the country reveal that 87% of pregnant women suffer from anemia and about 10% have severe anemia (Hb < 7 g/dl). Variations in the prevalence rates of anemia are seen within the country with the lowest prevalence of 33% being reported from Andhra Pradesh to the highest of 98% in Rajasthan[1]. A recent study on the prevalence and etiology of nutritional anemia in early childhood in an urban slum area of east Delhi indicated a high prevalence (76%) of anemia[2]. Earlier studies from the National Institute of Nutrition (NIN), Hyderabad showed an average anemia prevalence rate of 68% in preschool children[3].

## 1.2. Etiology of iron deficiency

Adolescents are vulnerable to iron deficiency because of increased iron requirements related to rapid growth. Iron needs are highest in males during peak pubertal development because of a greater increase in blood volume, muscle mass and myoglobin[4, 5]. In females, after menarche, iron needs continue to remain high because of menstrual blood loss, which averages about 20 mg of iron per month, but may be as high as 58 mg in some individuals[6]. In spite of increased iron needs, many adolescents, particularly females, may have iron intakes of only 10-11 mg/day of total iron, resulting in approximately 1 mg of absorbed iron[6]. About three fourths of adolescent females do not meet dietary iron requirements, compared to 17% of males[4]. Iron deficiency is an end result of a long period of negative iron balance, mainly due to poor dietary availability, rapid growth of the person, and blood loss. The pathological stages are[4]:

- *Pre-latent deficiency:* Liver (Hepatocytes and macrophages), spleen and bone marrow show reduced iron stores (reduced- bone marrow iron and serum ferritin).
- *Latent deficiency:* With very low or absent bone marrow iron stores, there is progressive reduction in plasma iron; the bone marrow receives little iron for hemoglobin regeneration (bone marrow iron is absent, serum ferritin is <12µg/L, transferrin saturation is <16% and free erythrocyte porphyrin is increased); however, hemoglobin concentration remains normal.

- *Iron deficiency anemia:* This is a very late stage of iron deficiency with progressive fall in hemoglobin level and mean corpuscular volume.

### 1.3. Consequences of Iron Deficiency Anemia

Iron is essential to all cells. Functions of iron include involvement in energy metabolism, gene regulation, cell growth and differentiation, oxygen binding and transport, muscle oxygen use and storage, enzyme reactions, neurotransmitter synthesis, and protein synthesis[7]. Iron has diverse biological functions. It is this diversity that accounts for the wide-ranging impact of its deficiency. Iron deficiency commonly remains unrecognized[8] as it tends to develop slowly and adaptation occurs over the time. Severe anemia is an important risk factor in pregnancy. Reports from India indicate that 16% of all maternal deaths are attributable to anemia[9]. Maternal anemia also contributes to an increase in perinatal mortality, low birth weight, and fetal wastage. Studies on low income pregnant women in India showed a three-fold greater incidence (34.5%) of premature deliveries in severely anemic women compared to the normal. Maternal immune depression and increase in morbidity has also been reported among anemic pregnant women[10]. In the past, several studies have shown that IDA often leads to irreversible impairment of the child's learning ability and other behavioral abnormalities[11]. The neurochemical roles of iron are not fully understood but it is clear that low levels of iron can have a significant adverse impact on brain function[11].

### 1.4. Management of IDA.

Iron-deficiency anemia remains prevalent despite the widespread ability to diagnose the disease and availability of medicinal iron preparations[12]. Various strategies including allopathic/ ayurvedic treatment have been used for curing IDA. Whereas, the allopathic treatment leads to various side effects along with the therapeutic effect like heartburn, nausea, upper gastric discomfort, constipation, diarrhoea[13] along with generation of damaging free radicals in the intestine[14], ayurvedic treatment is reported to be comparatively safer and free from side effects[15]. The present study is one step in such direction to find out the effectiveness of *Reetha bhasma* in the management of IDA. Many ayurvedic preparations containing iron are available in market including *Lauha bhasma* and *Mandoor bhasma.* Although they are effective in curing IDA, but the processing involves various complicated steps that leads to high cost of therapy[15]. However, the preparation of *Reetha bhasma* is comparatively easier[16], which may results in cost effective therapy. Thus, the present study is aimed at providing cost effective therapy against IDA, keeping in mind the majority of population and health issues as well.

## 2. LITERATURE REVIEW

### 2.1. Physiology of IDA

Iron is a critical element in human metabolism that is not excreted[17]. Under normal circumstances, iron absorption and iron loss are tightly coupled. Iron losses in the absence of bleeding, which occur through sweat, shedding of skin cells, and sloughing of gastrointestinal epithelial cells, amount to approximately 1 to 2 mg/day from a body store of approximately 4,000 mg. Menstrual losses can add another 30 mg/month[17], and foetal needs during pregnancy can require an additional total of 1,000 mg, leading to more frequent iron deficiency in women. Patients on hemodialysis can also lose excessive amounts of iron, averaging 2,000 mg/yr[18], invariably leading to IDA in the absence of replacement therapy. Total body iron stores are distributed into several compartments. In normal individuals, the circulating red blood cell compartment contains 1,800 mg in hemoglobin, the liver stores approximately 1,000 mg as ferritin, and macrophages contain another 600 mg. Muscle myoglobin accounts for approximately 300 mg, whereas the bone marrow stores another 300 mg. Only approximately 3 mg exists in the transport form, transferrin, in the circulation[18]. Iron deficiency can occur as a result of either inadequate absorption or excessive loss of iron, although gastrointestinal blood loss is the most common cause of iron deficiency anemia in men and postmenopausal women[19]. Iron is absorbed primarily in the duodenum and proximal jejunum, although the process is relatively inefficient. Heme iron, typically found in meat, is absorbed at a rate of about 30% of intake, whereas inorganic iron is absorbed at only a 10% rate of intake, producing a net absorption of 1 to 2 mg/d[20]. However, the inorganic absorption of iron can be up or down regulated in the duodenal wall through regulation of a number of iron transport proteins. On the luminal side, ferric (3+) iron is converted to ferrous (2+) iron by duodenal cytochrome b; subsequently, the divalent metal transporter 1 (DMT1) transports ferrous iron from the duodenal lumen into the intestinal epithelial cell, while dietary heme iron is transported by heme carrier protein 1(HCP1)[21]. Once inside the cell, heme oxygenase releases iron from protoporphyrin, presumably allowing it to enter the same pool as non-heme iron. On the basolateral side, ferroportin 1 (FP1) transports ferrous iron out of the cell, and hephaestin converts ferrous (2+) iron back to the ferric (3+) form when it enters the circulation and is bound by transferrin for transport (Fig 1) [22]. This transport mechanism can be regulated by 5 variables. First, duodenal epithelial cells themselves decrease absorption shortly after ingestion of large amounts of dietary iron (the dietary regulator). This is thought to be because of high concentrations of intracellular iron in the epithelial cell and its return to normal after a few days[23, 24].

Second, the "iron stores regulator" senses total body iron through unclear mechanisms and drives increased expression of DMT1. This leads to an increase in iron absorption by approximately 3-fold in iron deficiency or when there is inappropriate regulation of iron absorption such as in hereditary hemochromatosis[25]

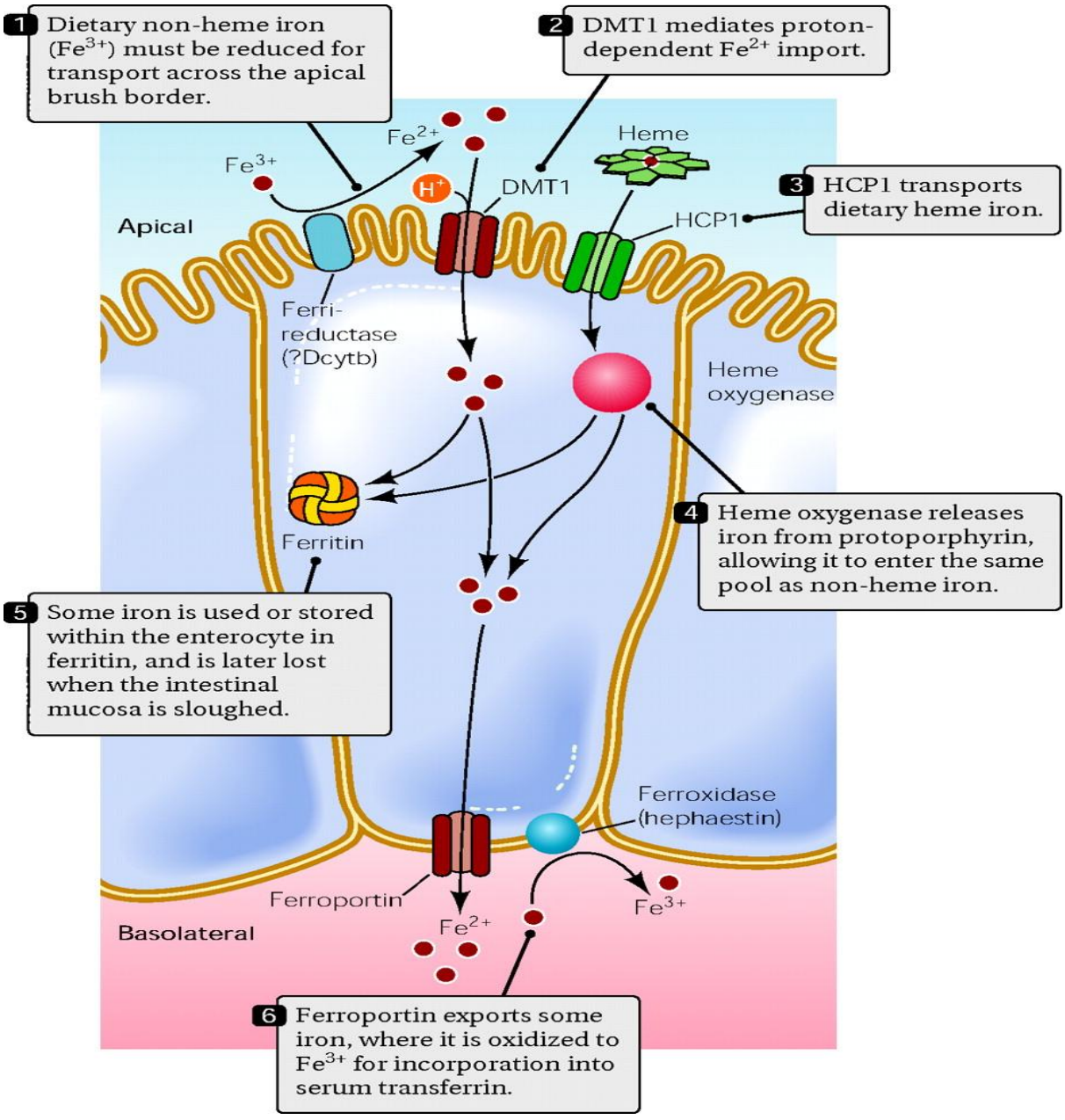
1 Dietary non-heme iron ($Fe^{3+}$) must be reduced for transport across the apical brush border.
2 DMT1 mediates proton-dependent $Fe^{2+}$ import.
3 HCP1 transports dietary heme iron.
4 Heme oxygenase releases iron from protoporphyrin, allowing it to enter the same pool as non-heme iron.
5 Some iron is used or stored within the enterocyte in ferritin, and is later lost when the intestinal mucosa is sloughed.
6 Ferroportin exports some iron, where it is oxidized to $Fe^{3+}$ for incorporation into serum transferrin.
$Fe^{2+}$
$Fe^{3+}$
$H^+$
DMT1
Heme
HCP1
Apical
Ferri-reductase (?Dcytb)
Heme oxygenase
Ferritin
Ferroxidase (hephaestin)
Ferroportin
Basolateral
$Fe^{2+}$
$Fe^{3+}$

**Fig 1: Intestinal iron absorption**

Third, an erythropoietic regulator increases iron absorption in response to rapid erythropoiesis and can increase iron absorption approximately 5-fold through mechanisms that have not yet been defined[22]. Acute hypoxia is a fourth factor that can acutely increase iron absorption[26,27] a fifth factor, chronic inflammation, appears to decrease the absorption of iron in the duodenum via DMT1 and FP1 downregulation[28,29,30]. This appears to be mediated through the iron regulatory hormone

hepcidin[31], which is induced during systemic infection, and is associated with decreases in DMT1 and FP1. Recent evidence suggests that hepcidin may also play a signaling role in regulating iron absorption in response to changes in iron stores[32]. However, even with maximal up regulation and adequate dietary supplementation, the duodenum can absorb a maximum of 5 to 7 mg/d, less than 0.2% of the total body iron stores [33, 34]. Thus, even small amounts of blood loss can create an iron deficiency state. Iron losses initially deplete the storage pool in the liver and macrophages, without causing an anemia. Continued iron losses produce a normocytic anemia without reticulocytosis. Further iron losses will produce classic hypochromic, microcytic anemia[35].

## 2.2. Iron Requirement

Absorption of iron is influenced by the amount of iron in the body; it decreases if individuals are iron replete and increases if they are deficient. Normally, men lose only 1 mg of iron every day (14 μg/kg/d), which is easily replenished through the diet. Such losses are proportionally less in women (0.7 to 0.8 mg/d). Menstrual bleeding causes an additional loss of 0.4 to 0.5 mg iron daily amounting to a total loss of 30 μg/kg/d. Women also lose iron to the placenta and the foetus, amounting to about 1.3 g of iron as the cost of normal delivery. Another critical period of increased iron requirements is early childhood and adolescence. During 6-24 months of age both physical growth and brain development occur at a rapid rate. In adolescence, there is a marked demand for iron to increase blood volume and muscle mass. As per ICMR report, the recommended dietary allowances of iron for Indians are; adult men: 28 mg/d, adult women: 30 mg/d, pregnant women: 38 mg/d, lactating women: 30 mg/d, boys (13-15 yr.): 41 mg/d, girls (13-15 yr.): 28 mg/d, and children (7-9 yr.): 26 mg/d[36].

## 2.3. Screening/Diagnosis of IDA

The Centers for Disease Control and Prevention (CDC) recommendations for screening adolescents for anemia suggest that all females be screened at least once every five years unless risk factors for anemia are present, resulting in the need for annual anemia screening. Adolescent males only need to be screened for anemia in the presence of risk factors. When iron status is adequate, iron stores and erythropoiesis remain normal. With iron depletion, stores are reduced while erythropoiesis is maintained. Iron deficiency is associated with depleted stores and abnormalities in iron metabolism and red blood cell biochemistry. Although there is no single laboratory test that specifically indicates iron deficiency anemia, several tests are used to determine iron status and the presence of anemia[4].

### *2.3.1. Hemoglobin/ Hematocrit*

Measurement of hemoglobin (Hb.) or hematocrit (Hct) is the most cost efficient and commonly used method to screen for anemia. Determining the concentration of hemoglobin, an iron-containing protein, in red blood cells is a more sensitive and direct

**Table 1: Cut off points for hemoglobin and hematocrit for diagnosis of anemia[4]**

| Gender/Age (yrs) | Hemoglobin<g/dL | Hematocrit<% |
|---|---|---|
| ***Children*** | | |
| < 5 | 11.0 | 33.0 |
| 5-11 | 11.5 | 34.0 |
| 12-14 | 12.0 | 36.0 |
| ***Females*** | | |
| 12-14.9 | 11.8 | 35.7 |
| 15-17.9 | 12.0 | 35.9 |
| 18+ | 12.0 | 35.9 |
| *Pregnant woman* | 11.0 | 33.0 |
| ***Males*** | | |
| 12-14.9 | 12.5 | 37.3 |
| 15-17.9 | 13.3 | 39.7 |
| 18+ | 13.5 | 39.9 |
| ***Laboratory Test Value*** | | |
| Ferritin | <15 μg/L | |
| Serum transferrin receptor concentration (TfR) | >8.5 mg/L | |
| Transferrin saturation | <16% | |
| Mean cell volume (MCV) | <82/85 fL* | |
| Red cell distribution width (RDW) | >14% | |
| Erythrocyte protoporphyrin (FEP) | >70 μg/dL | |

*** <15 yrs/>15 yrs of age**

**Severe anemia: Hemoglobin < 7g/dl**

**Very severe anemia: Hemoglobin < 4g/dl**

indicator of anemia than hematocrit (percentage of red blood cells in whole blood). With a low Hb/Hct, a presumptive diagnosis of iron deficiency anemia is supported by a response to iron therapy. If the Hb. level does not improve after taking iron supplements for one month, further assessment is indicated. The cut off points of Hb. & Hct. for anemia diagnosis are given in Table 1[4].

***2.3.2. Serum ferritin***

A low serum ferritin (<15 μg/L), in addition to a low hemoglobin or hematocrit, confirms the diagnosis of iron deficiency anemia. An intracellular iron storage protein, serum ferritin reflects iron reserves (1 μg/L= 8-10 mg stored iron) [31]. Ferritin is a highly conserved protein complex that plays an important role in iron storage and is recognized as the main iron-binding protein in non erythroid cells[37]. Intracellular ferritin is synthesized by the smooth endoplasmic reticulum. Serum ferritin is synthesized by the rough endoplasmic reticulum and glycosylated by the golgi apparatus before being secreted[37]. Generally, serum ferritin is directly related to intracellular ferritin and thus total body iron stores. Only iron deficiency causes very low serum ferritin concentrations; therefore a low serum ferritin concentration is very specific for iron deficiency[38].

### *2.3.3. Transferrin saturation*

An elevated serum transferrin receptor concentration (TfR) (>8.5 mg/L) is an early and sensitive indicator of iron deficiency. It is, however, also raised in Thalassemia and hemolytic anemia. Serum transferrin, an iron transport protein, increases when iron stores are low. TfR reflects the number of transferrin receptors on immature red cells, and thus the tissue iron need. Serum TfR concentration remains normal when chronic disease, inflammation or infection are present, distinguishing iron deficiency anemia from anemia of chronic disease. If iron deficiency anemia and anemia associated with chronic disease occur simultaneously, TfR concentration is elevated[38.]

## 2.4. Additional Screening

In adolescents who have iron deficiency which is not responsive to iron therapy, additional screening may be indicated to rule out the following[38]:

• Sickle cell trait and Thalassemia in adolescents who are African, African- American, African- Southeast

Asian or of Mediterranean descent.

• Parasitic infections (e.g., in newly arrived immigrants or travelers from developing countries).

• Anemia related to folate or vitamin B12 deficiency or infection or chronic inflammation.

## 2.5. Treatment of IDA

### *2.5.1. Primary prevention through diet*

Primary prevention of iron deficiency is achieved through proper dietary iron intake. Table 2 presents the recommended dietary allowances for iron. It should be noted that the iron requirements for vegetarians and vegans are approximately 1.8 times higher than for

omnivores because of the bioavailability of ingested iron[39]. Lean meats, especially beef, have high iron contents that are highly bioavailable. Non animal foods that are high in iron include nuts, seeds, legumes, bean products, raisins, dark green leafy vegetables, whole grains, and iron fortified cereals[40].

**Table 2: Recommended dietary allowances for iron[41]***

| | Males | | Females | |
|---|---|---|---|---|
| Age (y) | Omnivore | Vegetarian/ vegan | Omnivore | Vegetarian/vegan |
| 14-18 | 21 | 40 | 15 | 28 |
| 19-50 | 16 | 28 | 18 | 33 |
| ≥51 | 8 | 14 | 8 | 14 |

*Units for iron are shown in milligrams per day. Note that the iron requirements for vegetarians and vegans are roughly 1.8
times higher than omnivores because of the bioavailability of ingested.

#### 2.5.1.1. Absorption and bioavailability

Heme iron, found in meat, poultry, and fish, has a bioavailability of approximately 30% (i.e. 30% of ingested heme iron is absorbed). Non-heme iron, found in plants and iron-fortified foods, has a bioavailability of less than 10%. Iron in food is mostly ferric iron and is most soluble and best absorbed below a pH of 3. Ferrous iron, found in oral iron supplements, is soluble even at a pH of 7 to 8 and is more easily absorbed[42]. This may be important in patients with altered gastric environments such as achlorhydria (commonly affecting the elderly), gastric atrophy, and Helicobacter pylori infection[43]. Enhancers of iron absorption include heme and ascorbic acid or vitamin C (found in broccoli, cauliflower, and many fruits). Tannins (found in tea and coffee), phytates (found in bran, cereal grains, flour, legumes, nuts, and seeds), and calcium (found in dairy products and many over-the-counter antacids) inhibit iron absorption. Strategies to enhance iron absorption includes drinking tea and coffee 1 to 2 hours after, rather than with, a meal; eating foods with high vitamin C content during meals; consuming dairy products as snacks rather than during meals; and eating foods containing inhibitors during meals with lowest iron content[43].

### *2.5.2. Iron supplementation therapy (Allopathic treatment)*

Iron can be supplemented orally, intramuscularly, and intravenously. Blood transfusions are sometimes required in severe cases[44]. Many oral iron preparations are available, both in tablet and elixir form. These include ferrous sulfate, ferrous fumarate, and ferrous gluconate. Extended release, carbonyl iron and polysaccharide-iron complex formulations are also available. The ferrous salts, including ferrous sulfate, ferrous fumarate, and ferrous gluconate, are all equally tolerated and effective[45]. Of these,

ferrous sulfate is the cheapest and is widely recommended as first line therapy[45, 46]. Ferrous salts should be given on an empty stomach and should not be given within 2 hours of any inhibitor of iron absorption[44]. Some authors recommend taking ascorbic acid, 250 mg, along with ferrous salts to enhance absorption[44]. The most common complication of oral ferrous salt therapy is gastrointestinal upset, including abdominal pain, nausea, vomiting, and constipation. This can occur in 15% to 20% of patients receiving oral iron therapy[4]. Alternatives include using extended- release iron preparations[46], liquid iron preparations[48], ferrous salts with lower elemental iron contents[44], and taking iron tablets with meals[49]. Although the CDC recommends prescribing 60 to 120 mg/d of elemental iron[4], many authors recommend higher starting dosages, usually ferrous sulfate 300mg (60 mg elemental iron) 3 to 4 times daily[44, 49]. Elemental iron ingested at 200 to 300 mg/d will result in iron absorption of approximately 50 mg/d[4]. Iron can also be given parenterally, that is, as intramuscular (IM) and intravenous (IV) preparations[50]. Both may be appropriate for patients who cannot tolerate oral iron as well as for patients with severe gastrointestinal bleeding, malabsorption, or both. Parenteral iron is available as iron dextran (IM or IV), ferric gluconate complex (IV), and iron sucrose (IV). Side effects of parenteral iron include local reactions (pain, muscle necrosis, and phlebitis) as well as anaphylaxis, fever, urticaria, and flare of rheumatoid arthritis[44, 47]. Advanced methods of maintaining iron balance such as using recombinant human erythropoietin along with parenteral iron therapy is usually used in patients with renal failure[51].

### *2.5.3. Ayurvedic treatment*

*Lauha* & *Mandoor* are the classical Indian herbo-mineral products that have *Lauha bhasma* (incinerated iron) or *Mandoor bhasma* (Incinerated iron oxide) as one of their prime ingredients[15]. They are considered to be rejuvenators of the rakta dhatu (Blood) which itself is considered to be one of the seven essential factors that behold the very existence of the body and is said to be one of the ten abodes of the life mentioned in ayurvedic classics[15]. The herbo-metallic preparations along with their composition used in the treatment of anemia are listed in Table 3.

**Table 3: List of herbo-metallic preparations used for the treatment of anemia**[15]

| Name of the Drug | Ingredients | Indications | Reference |
|---|---|---|---|
| Navayasa Lauha | Amalakī, *Lauha bhasma* Yaṣti madhu, Amṛuta kvatha | Anaemia, Dyspepsia, Duodenal ulcer | Bhaisajya Ratnavalī |
| Rakta Pittantaka Lauha | Amalakī, Pippalī cūrṇa, *Lauha bhasma* | Anaemia & Bleeding disorders | Bhaisajya Ratnavalī |
| Saptamṛuta Lauha | Yaṣtimadhu, Triphala, *Lauha bhasma* | Anaemia, Diseases of eye, Oedema | Bhaisajya Ratnavalī |
| Shilajitvadi Lauha | Suddha Silajatu, Trikaṭu, Makṣika *bhasma*, *Lauha bhasma* | Anaemia | Bhaisajya Ratnavalī |
| Tapyadi Lauha (without silver) | Triphala, Trikaṭu, Trimada, Suddha Silajīta, Svarṇamakṣika *bhasma*, *Lauha bhasma*, | Anaemia ,Urinary disorders, Hepatic disorders, Oedema | Rasatantra Sara |
| Tapyadi Lauha (with silver) | Triphala, Trikaṭu, Trimada, Suddha Silajīta, Svarṇamakṣika *bhasma*, *Lauha bhasma*, | Urinary disorders, Hepatic disorders, Anaemia, Oedema | Rasatantra Sara |
| Viṣama Jvarantaka Lauha | Cīrayata, Pittapapḍa, Devdarū, Pṛiṣthaparṇī, Trikaṭu, Trayamaṇa, Giloya, Abhraka *bhasma* | Anaemia , Chronic fevers, Malabsorption syndrome. | Bhaisajya Ratnavalī |
| Punarnavadi Maṇḍūra | Punarnava, ṣaḍūṣaṇa, Triphala, Danti, Nisotha | Anaemia, Worm infestation, ,Inflammatio ns, Digestive diseases | Bhaisajya Ratnavalī |

#### 2.5.3.1. *Bhasma*

*Bhasmas* are the unique ayurvedic metallic/ mineral preparations, treated with herbal juices or decoctions, and exposed for certain quantum of heat as per *Puta* system of Ayurveda[52]. During the preparation of *bhasmas*, metallic/ herbal substances are

subjected to the process of incineration and reduction to ash. *Bhasma* preparation involves various steps which are discussed below.

#### 2.5.3.1.1. Method of preparation of *Bhasma*

##### *2.5.3.1.1.1. Shodhana*

*Shodhana* is the process through which the external and internal impurities of metals and minerals are removed. Chemical purification is different from medicinal purification. In chemical purification it is only elimination of foreign matters, whereas in medicinal purification the objectives involved are:

- Elimination of harmful matter from the drug
- Modification of undesirable physical properties of the drug
- Acceleration of some of the characteristics of the drug
- Enhancement of the therapeutic action

There are two kinds of *shodhana*. The first type, *samanya shodhana* (general purification), is applicable to the large number of metals or minerals as heating the thin sheets of metals and immersing them in oil (*taila*), extract (*takra*), cow urine (*gomutra*), and other materials. The second type, *Vishesha shodhana* (special purification), is applicable only to specific metals, minerals, and in certain preparations. *Vishesha shodhana* includes *bhavana*, *svedana*, *nirvapana*, and *mardana*[53]. After *shodhana*, *bhasmas* become soft and malleable for further processing and their metallic property is improved. The main apparatus required includes *dola yantra*, *khalva yantra*, and *musha yantra*[54]. When mineral drugs are heated in a furnace in the presence of *dravaka*, substances (liqueficants) like alkali and acid release their *satva*. This is the purest form of any herbal or mineral drug[53]. All the metals except mercury are found in nature in solid state, and they all fuse under high temperature to attain a liquid state. When the temperature lowers they again return to their natural physical form (i.e., in the solid state). But these fused metals in the presence of some liqueficants do not return into their natural solid state even when the temperature lowers (i.e., the metals remain in liquid form). This method of obtaining metals in liquid form is called *dravana* and the obtained liquid metal is called *druti*. *Druti* holds superior character with respect to efficacy, toxicity, and increased shelf life than its native metals and retains its fluidity for a longer time with proper preservation.

##### *2.5.3.1.1.2. Marana*

*Marana* is essentially the burning process or calcination. The purified metal is placed into a mortar and, with a pestle, ground with the juice of specified plants or *kashayas*, mercury (in metallic state), or a compound of mercury such as mercury perchloride

(*sauviram*), mercuric subchloride (*ras karpur*), cinnabar (*ingalekam*), or an amalgam of sulfur and mercury (*kajjali*) for a specified period of time. The metal that is intended for *marana* is known as a primary metal (*pradhan dhatu*); the other metal, which is taken in small proportions for the *marana* of the primary metal, is known as secondary metal (*sahaya dhatu)*[53]. Small cakes (*chakrikas*) are made with the ground paste of the minerals and dried under the sun. The size and thickness of the cakes depend on the heaviness of the drug and size. The heavier the drug, the thinner the cakes. These cakes are dried well under the shade and placed in one single layer in a mud tray (*sharava*) and closed with another such tray; the clay-smeared cloth keeps both the lid and the container in a position. The clay-smeared cloth is applied seven times and dried to seal the crucibles properly. A pit is dug in an open space and half the pit is filled with dried cow dung cakes. The crucibles are placed in the half-filled pit and are covered with cow dung cakes up to the brim of the pit. Fire is then ignited on all four sides and in the middle of the pit. When the burning is over, the contents are allowed to cool completely on their own. *Marana* differs with the nature of the substance to be calcinated. For example, organic substances such as herbs are burnt in open air, whereas inorganic substances such as metals like *rajata* (silver) are burnt in closed containers. In either case the end product is a *bhasma* of substance taken for *marana*. For example, the end product in the case of silver (*rajata*) is called as *rajata bhasma*. *Marana* of inorganic substances is called *puta* and the process of *marana* of herbs in closed freshly made containers is known as *puta paka*[55]. *Bhasmas* obtained by *marana* from primary metals together with herbs (*mulika*) are called *mulika marita bhasma*; the ones where the second metal is taken for the *marana* of primary metal are called *parada* (mercury) *marita,* or *talaka* (arsenic trisulphide) *marita bhasma,* depending upon the second metal used for the purpose. During the process the second metal would finally volatilize itself at the temperature of *marana*, leaving behind the *bhasma* of primary metal. Very few metals like copper or iron still bear some impurities after the *marana*. In such cases the whole process is repeated until a purified and therapeutically safer product for internal use is obtained. In addition, a process called *amritikarana* is done to make these metals safer[54]. The process consists of heating the product from the *marana* procedure in the presence of some herbal materials to improve safety and therapeutic effect. In this process the required amounts of *triphala* decoction, cow's *ghritika*, and *dhatu bhasma* are placed in an iron pot. Mild heat is applied until the medicinal fluids are completely evaporated. *Bhasma* that remains at the end of this process is safer and possesses higher therapeutic efficacy.

#### 2.5.3.1.2. Quality Control of *Bhasma*

Traditionally, the end points of incineration of a metal and its conversion to a *bhasma* state are evaluated based on the following criteria[52]:

1. There should be no *chandrika* or metallic lusture (*nischandrika*).
2. When a *bhasma* is spread between the index finger and thumb, it should be so fine as to get easily into the lines and crevices of the fingers (*rekhapurita*).
3. When a small quantity is spread on cold and still water, it should float on the surface (*varitara*).
4. The *bhasma* should not revert to the original state (*apurnabhava*).

**2.6. Monitoring iron status and duration of therapy**

At a minimum, the hemoglobin concentration should be checked 3 to 4 weeks after beginning oral iron supplementation therapy. If a patient with IDA takes ferrous sulfate 300 mg (60 mg elemental iron) 3 to 4 times daily, the hemoglobin concentration should rise approximately 2 g/dL after 3 to 4 weeks[44, 48]. If the rise in hemoglobin concentration is less, this may be due to poor compliance, misdiagnosis, malabsorption[48], coexisting cause of anemia in addition to IDA (eg, anemia of chronic disease, thalassemia), or continued blood loss[44]. If there is an appropriate response in hemoglobin concentration after 1 month, oral iron supplementation should be continued until the hemoglobin concentration normalizes. This will depend on the degree of anemia and the rate of response to therapy. For patients who are at risk for or have had gastrointestinal bleeding, some authors recommend stopping iron supplementation once the hemoglobin concentration has normalized so that if there is further occult bleeding, this will cause IDA and can be quickly detected[44]. Others recommend continuing therapy until iron stores are replenished. This can take from 3[49]- 6 months[44, 47].

**2.7. Description of *Sapindus mukorossi* (Reetha)**

*Sapindus mukorossi*, popularly known as soapnut, soapberry, washnut, ritha, aritha, dodan, doadni, doda, kanma and thali belongs to Family Sapindaceae, Kingdom Plantae, Subkingdom Tracheobionta (Vascular plants), Division Magnoliopsida (Flowering plants), Class Magnoliopsida (Dicotyledons), Subclass Rosidae,

Order Sapindales and Tribe Andropogoneae. The major parts used include fruits and shells of the soapnut[56]. The tree grows in temperate to tropical regions throughout North India and Nepal in hills and plains in deep clayey loam soil with areas experiencing nearly 150 to 200 cm of annual rainfall. Reetha tree is basically an attractive medium sized deciduous tree which stands up to 20 m in height, with gray smooth bark and pinnate leaves. The tree bears leaves in 5-10 pairs, with large drupes. The trunk of the

tree is straight and cylindrical, going 13-16 ft in height and has an umbrella-like hemisphere measuring about 16 ft in diameter. The tree is ever-growing and in 70 years of existence, it can attain a height of up to 82 ft and a girth of up to 9-16 ft. The size of the leaflets tapers towards the tip of the rachis. The flowers on Reetha plant grow during the summer season and are small in size and greenish white in colour. The fruit appears in July and August and ripens by the months of November and December. This ripened fruit is then either sold in the market as soap nut or collected for seeds, as they tend to germinate easily. The dried fruit has a soapy texture and is used to prepare quality shampoos, detergents and a substitute for washing hands. Moreover, the plant is soft and green when it is fresh[57].

### *2.7.1. Uses of Sapindus mukorossi*

1. Reetha has been placed in the list of herbs and minerals in Ayurveda. Reetha is used as the main ingredient in soaps and shampoos for washing hair, as it is considered good for the health of hair and for washing woollen clothes. This is why some botanists have named the species as *Sapindus detergens*[57].
2. When used topically, it is found to be effective in the treatment of eczema, psoriasis, and for removing freckles. Soapnut is also considered as a natural exfoliant[57].
3. When prepared by an ayurvedic doctor it can treat chlorosis and epilepsy. It is also used as an expectorant

for severe lung congestion, and can help to promote blood circulation in patients with low blood pressure[57].

4. It can also be prepared as a digestive aid, an anti-venom, or to treat diarrhoea, cholera and paralysis[57].
5. Saponins in soapnuts have been found to inhibit tumour cell growth in humans[58.]
6. The solution made from the fruits of *Sapindus* species has been found to decrease behaviours associated with migraine in mice[59.]
7. Hepatoprotective activity[60].
8. Anti-platelet Aggregation activity[61].
9. The plant is known for its antimicrobial properties that are beneficial for septic systems[62].
10. Reetha is reported to be used as abortifacient[63].
11. There have been clinical trials on the use of *Sapindus mukorossi* as a spermicide (replacing Nonoxynal-9, which has shown to lead to widespread sexually transmitted infections.) [64]

12. Reetha is rich source of iron[57]. However, because of presence of saponins, it acts as hemolytic. If, prepared by special procedure, it can be beneficial in the treatment of anemia[57]. But no research has been reported yet.

***2.7.2. Ayurvedic properties of Sapindus mukorossi*** [65, 66]

| | | |
|---|---|---|
| Guna (Qualities) | : | Laghu (light), tikshna (sharp) |
| Rasa (taste) | : | Tikta (bitter), katu (pungent) |
| Vipaka (post digestive taste) | : | Katu (pungent) |
| Virya (potency) | : | Ushna (hot) |
| Prabhava (effect) | : | Vaman (emesis) |

## 3. RATIONALE OF THE STUDY

Iron deficiency and consequent anemia (IDA) has a very high global incidence since it poses serious health problems like general weakness, lethargy, lassitude, sub-optimal work performance and in certain situations mental retardation, poor intelligence and abnormal immune response[67]. Various treatment strategies including allopathic/ ayurvedic medicines can be used for the treatment of IDA. Innumerable iron containing allopathic formulations are available in the market for treatment of iron deficiency anemia containing one or other iron salts like, ferrous sulphate, ferrous fumarate, ferrous glycine sulfate, ferric hydroxide polymaltose complex, ferric ammonium citrate, iron dextran, iron choline citrate, iron sorbitol citrate, ferrous calcium citrate, ferrous gluconate, colloidal iron hydroxide, ferrous succinate and ferric hydroxide[58]. However, the long term treatment with iron salts is associated with several side effects like heartburn, nausea, upper gastric discomfort, constipation and diarrhea[13]. Recently it has been shown to generate damaging free radicals in the intestine[14]. An alternative approach to therapy is to enhance the absorption of dietary iron, rather than increase iron in the diet. A variety of such substances have been marketed, viz. surface acting agents, carbohydrates, inorganic salts, amino acids and ascorbic acid. None of these have become popular, although ascorbic acid has been proved to be effective[68]. The Indian system of medicine - Ayurveda, also offers a large number of iron preparations which have been used for centuries containing *Lauha bhasma* and *Mandoor bhasma* as the main ingredients.

Despite the availability of a large number of iron salts and preparations for correction of anemia and iron deficiency, the need of a better preparation has always been felt. The

present research is undertaken in order to prepare the *bhasma* of *Sapindus mukurossi* (Reetha) and to check its potential in curing IDA. The effectiveness of *R*eetha *bhasma* in treating IDA can be compared with the existing ayurvedic *bhasmas*. Reetha fruits are rich source of iron. However, the research on the iron content determination has not been performed yet. Reetha fruits, if used in raw form, have been found to show emetic activity[64]. So as to make the fruits easily administrable, *bhasmikaran* is required. In *bhasmikaran*, the fruits are subjected to the process of incineration and reduction to ash. The *bhasmikaran* of the fruits offers the following advantages which makes the fruits administrable.

1. Elimination of harmful matter from the drug: Reetha fruits contain triterpenoidal saponins[69] as chemical constituents which are not desired for the hematinic activity as the saponins are hemolytic in nature[70]. When the fruits are incinerated at high temperature it may result in destruction of saponins[71]. Thus, the required iron may get concentrated and the undesirable constituents may be removed from the *bhasma*.
2. Conversion of some of the characteristics of the drug to different stages: Iron present in the free form gets converted into the iron oxides after *bhasmikaran* which results in improved iron absorption.
3. Enhancement of the therapeutic action: As the iron gets concentrated after *bhasmikaran*, the potency of the *bhasma* increases resulting into dose reduction.
4. After *bhasmikaran*, the particle size of herbal drug reduces to such an extent as that of nanoparticles[72], which ultimately results into a formulation which distributes readily into body and is easily absorbable. The significance of using *R*eetha *bhasma* in comparison to the available iron *bhasmas* include:

- Easy availability of the raw drug.
- Easy processability: As per the Ayurveda, the guna (quality) of Reetha is laghu (light) [65], so less processing steps are required during *bhasmikaran* in comparison to preparation of other iron *bhasmas*, thereby, making the formulation cost effective.

Thus, the present research aims at the preparation of the economical *bhasma* used in the management of IDA without compromising therapeutic efficacy.

## 4. PLAN OF THE WORK

The whole study has been divided into following parts.

**First year**

1. Exhaustive literature survey---------- 3 months
2. Procurement of raw herb---------- 3 months

3. Authentication of drug---------- 1 month
4. Method development for analysis of iron ---------- 3 months
5. Determination of iron content in powdered drug.-------- 2 months
    - Colorimetric determination
    - Atomic absorption spectroscopy

**Second year**

6. Preparation of *Reetha bhasma* using different methods.
    - Direct *bhasmikaran*
    - Conventional *bhasma* preparation method
    - *Shodan*a by using lemon juice, onion and carrot juice.
7. Evaluation of *bhasma* using various tests.
8. Determination of iron content in *bhasma.*
    - Colorimetric determination
    - Atomic absorption spectroscopy

**Third year**

9. Comparison of emetic activity of raw herb as well as *bhasma.*
10. To check raw drug as well as *bhasma* for the presence of saponins- To test raw drug as well as *bhasma* for the hemolytic activity.
11. To prepare combinations of *bhasma* with different herbs and to check the efficacy against curing anemia (Turmeric, punernava, triphla, bahera).
12. *In vitro* dissolution studies of prepared *Reetha bhasma*.
13. Comparative study of iron content from *Reetha bhasma, Lauha bhasma* and *Mandoor bhasma.*
14. *In vivo* determination of iron from *Reetha bhasma* combination with best dissolution release profile.

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